Polycyclic Aromatic Hydrocarbons in Carcinogenesis

David Warshawsky

Department of Environmental Health, University of Cincinnati, Cincinnati, OH 45267-0056 USA

A symposium on "Polycyclic Aromatic Hydrocarbons (PAHs) in Carcinogenesis" was presented at the third International Congress of Pathophysiology held in Lathi, Finland, 28 June–3 July 1998. The congress was also sponsored by the International Union of Biological Sciences and the International Society of Free Radical Research. Institutional support for the symposium included the Electric Power Research Institute, National Center for Toxicological Research, and EPA/National Health and Environmental Effects Research Laboratory and the Office of Solid Waste and Emergency Response. The symposium focused on the sources, carcinogenicity, genotoxicity, and risk assessment of individual and mixtures of PAHs that are found in solid wastes, Superfund sites, and other hazardous waste sites. Based on the occurrence of PAHs at numerous Superfund sites and the significant data gaps on the toxic potential of certain PAHs, the information developed during this symposium would be of value in assessing health risks of these chemicals at Superfund and other hazardous waste sites.

Seven lectures were presented at the symposium. The first presentation dealt with sources of PAHs and those that were most commonly found on the National Priority List (NPL) (*1*). The second presentation discussed the mechanisms of activation of PAHs and *N*-heterocyclic aromatics (NHAs) in target tissues. The next three lectures dealt with synthetic and natural mixtures. The first of these reported on the relationship between synthetic mixtures and lung cancer in the mouse. The second lecture dealt with the tumorigenicity in mice fed coal tar or benzo[*a*]pyrene (B[*a*]P). The last of these lectures dealt with genotoxicity studies with reconstituted and field-derived PAH mixtures. The final two lectures were concerned with risk assessment of PAHs; the first was on the risk of coal tar mixtures and the second on the risk of environmental samples of PAHs.

Most U.S. hazardous waste sites listed on the NPL (Superfund or NPL sites) are contaminated with complex mixtures of chemicals that have migrated over time into several environmental media. To date, 1,410 sites have been listed or are proposed for listing on the NPL. PAHs have been identified as contaminants of concern at 352 of these sites and have been found in soil, groundwater, and sediment. Routine sampling of media for 16 PAHs containing from two to six aromatic rings occurs at sites with suspected PAH contamination. PAHs are commonly found at manufactured gas plant sites, wood-treating facilities, municipal and industrial landfills, manufacturing and chemical plants, and military and other federal facilities. B[*a*]P is the most commonly identified PAH at manufactured gas plant (MGP) sites. B[*a*]P is the most frequently identified PAH in all media (247 sites), in soil (198 sites), and in sediment (54 sites). Naphthalene is the most frequently occurring PAH in groundwater (125 sites).

The sources of PAHs were followed by a discussion of the formation of DNA adducts and oncogene-activating mutations by environmental pollutants (in particular, NHAs) that are found in complex mixtures. Complex mixtures that are produced from the combustion of organic materials have been associated with increased cancer mortality. These mixtures, such as tobacco condensate, automobile exhaust, and effluents from coal combustion contain homocyclic and heterocyclic PAHs. To better assess the contribution of NHAs to the carcinogenic potency of complex mixtures and to develop biomarkers of the carcinogenic process, studies were undertaken to assess the metabolic activation of NHAs. In particular, 7H-dibenzo[*c,g*]carbazole (DBC) and dibenz[*a,j*]acridine (DBA) were selected for study as model compounds based on animal testing. DBC and DBA were carcinogenic in more than one tissue. Both compounds are symmetric around their C_2 axis; the only difference is that the middle ring for DBC has a pyrrole ring, whereas DBA has a pyridine ring.

DBC has both local and systemic effects in the mouse; it is a potent skin and liver carcinogen following topical application and a lung carcinogen following intraperitoneal injection. DBC–DNA adducts are formed through phenols or directly through radical cations. In addition, the DBC–DNA adduct pattern consisting of seven adducts based on the ^{32}P-postlabeling technique is target-organ specific for lung, liver and skin. As a result of DBC exposure, mutations in H-*ras* oncogene in skin and liver tumors are found exclusively in the second base of codon 61 by A to T transversions. Similarly, the majority of mutations in K-*ras* in lung tumors in the third base of codon 61 are A to T transversions, whereas mutations in the second base are A to T transversions with a few A to G transitions.

In contrast, DBA is a moderate mouse skin carcinogen following topical application and a lung carcinogen following subcutaneous injection. DBA is metabolized to *trans*-dihydrodiols. DBA–DNA adducts are formed in skin through an active metabolite, the dihydrodiolepoxide, which binds to deoxyadenosine and deoxyguanosine residues. Mutations in the H-*ras* gene in skin tumors as a result of DBA exposure are found in codons 12, 13, and 61. These mutations involve G to T and A to T transversions in the second base and are consistent with the DNA binding of DBA.

These results indicate that although the two compounds are structurally similar, differing by only one carbon in the middle ring, there are major differences in their metabolic activation, including metabolism, DNA binding, mutation, and carcinogenesis. The biological differences for DBC and DBA are reflected in target-organ-specific proximate and mutagenic metabolites, DNA adduct patterns, and the resulting mutational spectra.

It was indicated that although some environmental hazards involve significant exposure to only a single compound, most instances of environmental contamination involve concurrent or sequential exposure to a mixture of compounds. Multichemical exposures are ubiquitous in air, water, and waste sites; therefore, these exposures are an important component of risk assessments. Although the individual components in a mixture may each induce toxic effects, they may also interact with the host organism in a manner that increases or decreases the observed toxic effects of each component. The default assumption that the EPA uses for assessing the risk for chemical mixture exposure is to assume that there are no interactions and that the toxic response observed is the additive sum of the toxic responses of each component.

This default assumption in cancer risk assessment was tested using a mixture of five environmental and carcinogenic PAHs: B[*a*]P, benzo[*b*]fluoranthene, dibenz[*a,h*]-anthracene, 5-methylchrysene, and cyclopenta[*c,d*]pyrene. Using lung tumors in strain

Address correspondence to D. Warshawsky, Department of Environmental Health, University of Cincinnati, PO Box 670056, Cincinnati, OH 45267-0056 USA.

Received 9 October 1998; accepted 24 November 1998.

A/J mice as a measure of tumorigenic activity and response surface modeling as the analytic method, we found that less than additive and greater than additive interactions were dose related. Response surface analysis produced statistically significant values for 10 interaction parameters. All of the binary interaction functions were antagonistic and were dominated by dibenz[*a,h*]anthracene. The response surface model closely predicted the observed lung tumorigenic responses of all the quintary mixtures as well as the dose–response curves of each individual PAH component. Although there were significant interactions, the extent of these interactions was limited (approximately twofold) and the EPA default assumption within the bounds of the study was supported and, therefore, these interactions probably will not have a major influence in the risk assessment.

In lifetime feeding studies with female B6C3F$_1$ mice, environmental coal tars were tumorigenic in the small intestine, liver, lung, and forestomach. Coal tars also induced sarcomas of skin and histiosarcomas and hemangiosarcomas in various organs. In contrast, B[*a*]P induced tumors in the tongue, larynx, esophagus, and forestomach. Because the PAHs in general and B[*a*]P in particular are thought to play a role in carcinogenesis by coal tars, studies were conducted to compare the effects of coal tars to a single component (B[*a*]P) within the tars.

Using the ^{32}P-postlabeling assay, DNA adduct levels were measured in the lung, liver, and forestomach of B6C3F$_1$ mice fed coal tar or B[*a*]P for 4 weeks. All three tissue sites demonstrated dose-dependent increases in DNA adduct levels, indicating that both B[*a*]P and coal tar PAHs were transported to the organ sites, even though no lung or liver tumors were found in the B[*a*]P-treated mice. The nature of the coal tar adducts in lung was investigated further by extracting the radioactive area of the thin-layer chromatography (TLC) plate corresponding to 10β-(deoxyguanosin-N^2-yl)-7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (N^2-dG-BPDE), the major adduct formed by B[*a*]P, and eluting through a column containing a monoclonal antibody to N^2-dG-BPDE. Adducts from lung DNA from B[*a*]P-treated mice were bound to the column, extracted, and produced a single HPLC peak. In contrast, in coal-tar-treated mice, approximately half of the radioactivity in the N^2-dG-BPDE area on the TLC plates did not bind to the antibody. Extraction of the remaining radioactivity from the column produced a number of HPLC peaks, suggesting multiple adducts. Because the antibody has specificity for other PAH adducts, the results indicate that additional adducts were formed in coal-tar-treated mice and may participate in processes leading to tumor formation.

B[*a*]P- and coal-tar-induced mutagenesis in the K-*ras* oncogene in forestomach tumors were also investigated. Both coal tar and B[*a*]P induced mutations in guanine at positions 1 and 2 in codons 12 and 13. Mutations at guanine were also found in lung tumors, but not in tumors of the small intestine in coal-tar-treated mice. Therefore, K-*ras* mutations may play a role in lung and forestomach tumors, but are not involved in small intestine tumors.

B[*a*]P dominates the cancer risk estimate when its concentration is >6,300 ppm in the coal tar mixture. In this case, the most sensitive tissue site is the forestomach. If the B[*a*]P concentration is <6,300 ppm, the more likely case, then lung tumors provide the largest estimated upper limit of cancer risk in humans exposed to coal tars. A multistage model was fit to the data and gave the relationship as follows: 1) risk $<2.55 \times$ percentage of coal tar in diet, or 2) risk $<2.55 \times 10^{-4}$ ppm coal tar in diet.

Coal tars aged in the soils may not be completely bioavailable to organisms when ingested or absorbed through skin. The bioavailable dose under these exposure conditions is less than would be calculated from exhaustive chemical extraction and characterization of PAH-contaminated media.

B[*a*]P alone did not account for all tumors induced by coal tars. Tumorigenesis by coal tars appears to reflect the contribution of chemicals other than B[*a*]P or reflect the modification of B[*a*]P activity by other chemicals in the coal tar. The data suggest that, for risk estimation, regulatory approaches based solely on the B[*a*]P content of complex mixtures of PAHs, such as coal tars, should be reevaluated. B[*a*]P does not appear to be responsible for the formation of tumors in the most sensitive site, lung tumors, when the concentration of B[*a*]P is <6,300 ppm in the coal tar mixture.

PAHs are major contaminants of oily and MGP sites. A study was undertaken to investigate the *in vivo* carcinogenicity and *in vitro* genotoxicity of crude MGP-derived PAHs, B[*a*]P, and a reconstituted mixture of MGP–PAH hydrocarbons. Carcinogenicity studies in juvenile male B6C3F$_1$ mice demonstrated that the hepatocarcinogenicity of MGP residues is primarily due to compounds other than the hydrocarbons. This was also confirmed in mutagenicity and fractionation assays, which suggest that alkyl PAHs may be important components of these mixtures.

The risk assessments of PAH at the EPA are carried out according to the *Guidelines for the Health Risk Assessment of Chemical Mixtures* (*2*) and the *Guidelines for Carcinogen Risk Assessment* (*2*). Proposed revisions to the cancer guidelines depart from the former system of alphanumeric weight-of-evidence categories in favor of a narrative classification statement. There is emphasis on the use of data other than tumor incidence in making judgments about the relevance of cancer observations (in animals or from specific exposure scenarios) to human risk assessment. Assessment of the carcinogenicity of a material under the revised guidelines is heavily dependent on a consideration of the mode of action by which it causes the carcinogenic effects. Mode of action informs both the hazard identification and dose–response processes. B[*a*]P mode of action can be characterized in this manner: 1) It is metabolized to reactive intermediates (including but not limited to diol-epoxides) in various tissues of the body; 2) these intermediates bind covalently to DNA or cause damage in other ways; 3) covalent binding to DNA can result in mutations in critical genes and thus initiate the carcinogenic process; 4) B[*a*]P is likely to serve as its own promoter; and 5) B[*a*]P may affect other stages of the carcinogenic process such as inducing metabolism or decreasing immune surveillance.

A revised hazard identification for B[*a*]P considers the following: 1) B[*a*]P produces tumors in every animal model in which it has been applied; 2) B[*a*]P produces tumors by all routes that have been tested; 3) B[*a*]P produces tumors at multiple sites, including sites distant from the point of exposure; and 4) some B[*a*]P-containing mixtures are known human carcinogens and many more are known animal carcinogens.

Revised hazard characterizations for B[*a*]P (and perhaps for other individual PAHs and for mixtures containing PAHs) indicate that these materials should be treated as if they are known human carcinogens. Furthermore, B[*a*]P (and perhaps others) should be considered carcinogenic by all routes. A hazard characterization of B[*a*]P should consider that B[*a*]P exposure to humans is primarily through inhalation, ingestion in food, and through dermal contact.

In summary, to determine appropriate clean-up actions for sites, risks must be evaluated from exposures to all contaminants of concern in all relevant media. In assessing the cancer risks from complex chemical mixtures found at Superfund sites, the EPA's hazardous waste program assumes that the risks due to individual chemicals are additive. In evaluating cancer risks from PAHs, the waste program currently analyzes the cancer risks of B[*a*]P and six other carcinogenic PAHs, using B[*a*]P as the reference

compound. Potential carcinogenic risks of other PAHs are generally not considered. Recent results of oral carcinogenicity studies of B[*a*]P in female mice and two separate mixtures of MGP residues indicate that new approaches to evaluating the cancer risks of complex PAH mixtures should be considered. Additional research is also needed, including toxicity/carcinogenicity studies of individual PAHs and PAH mixtures, bioavailability studies of individual PAHs and PAH mixtures in environmental media, and toxicity studies of environmental degradation products of PAHs.

Furthermore, carcinogenicity is the signature end point, but data are limited for other health effects. Studies should be conducted to allow assessment of immunotoxicity, developmental toxicity, and reproductive effects of PAHs. Quantitative assessment for B[*a*]P should use a low-dose linear approach based on the strong evidence for a mutagenic mode of action including irreversible changes. Appropriate physiologically based pharmacokinetic models should be applied to arrive at target doses. It should be possible to extend the observed range of the B[*a*]P dose response using nontumor data such as specific DNA adducts or mutation frequencies.

References and Notes

1. U.S. EPA. Description of NPL information documents. Available: http://www.epa.gov/superfund/resources/nplinfo/nplinfo.htm [cited 1 July 1998].
2. U.S. EPA. Risk Assessment Guidelines of 1986. EPA/600/8-87/045. Washington, DC:U.S. EPA, 1987.

Speakers

Dorothy Canter (Session Chairperson)
U.S. EPA
Office of Solid Waste and Emergency Response
Washington, DC

David Warshawsky (Organizer and Session Chairperson)
Department of Environmental Health
University of Cincinnati, Cincinnati, Ohio

Stephen Nesnow
U.S. EPA
National Health and Environmental Effects Research Laboratory
Research Triangle Park, North Carolina

Sandra Culp
Division of Biochemistry Toxicology
National Center for Toxicological Research
Jefferson, Arkansas

Steven Safe
Texas A & M University
College of Veterinary Medicine
College Station, Texas

Lawrence Goldstein
Electric Power Research Institute
Palo Alto, California

Rita Schoeny
Health and Environmental Criteria Division
U.S. EPA, Washington, DC